

CHLOROPHYLL MUTATIONS IN LENTIL

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ABSTRACT

A comparative assessment on frequency and spectrum of chlorophyll mutations induced by ethylmethane sulphonate (EMS) and hydrazine hydrate (HZ) was carried out in M_2 generation in lentil var. Pant L-406. Four types of chlorophyll mutants, albina, xantha, chlorina and viriscent were observed in the field when seedlings were 8-20 days old. Frequency of occurrence of chlorina mutants was relatively high in both mutagens followed by albina, xantha and viriscent. Chlorophyll mutation frequency increased with increasing concentrations of both mutagens. EMS induced higher frequency of chlorophyll mutations than HZ. Higher effectiveness was recorded in HZ treatments as compared to EMS. EMS was found to be a more efficient mutagen than HZ. Mutagenic effectiveness and efficiency recorded were higher at low concentrations of both mutagens.

KEY WORDS: Chemical mutagens, Chlorophyll mutants. *Lens culinaris*

INTRODUCTION

Induced mutagenesis serves as an important tool for creating usable genetic variability in a large number of crop plants in relatively short period of time. The chlorophyll mutation frequency is useful in assessing the potency of a mutagen. Hence, scoring of chlorophyll mutations has proved to be a much dependable index for evaluating the genetic effects of the mutagenic treatments. Several authors have reported the occurrence of different types of chlorophyll mutants in different crop plants such as chickpea (Kharakwal, 1998), mungbean (Khan *et al.*, 1998), urdbean (Singh *et al.*, 1999), cowpea (Ahmed John, 1999) and grasspea (Kumar and Dubey, 1998; Das and Kundagrami, 2000; Waghmare and Mehra, 2001). Mutagenic effectiveness is an index of the response of a genotype to the increasing doses of the mutagen, while mutagenic efficiency indicates the extent of genetic damage recorded in M_2 generation in relation to the biological damage caused in M_1 . In the present study, chlorophyll mutation frequency, effectiveness and efficiency of the mutagens was estimated in lentil var. Pant L-406 using ethylmethane sulphonate (EMS) and hydrazine hydrate (HZ) in M_2 generation.

MATERIALS AND METHODS

Uniform and healthy seeds of lentil (*Lens culinaris* Medik) var. Pant L-406, presoaked in distilled water for 9h, were treated with two chemical mutagens, viz., ethylmethane sulphonate (EMS) and hydrazine hydrate (HZ) for 6 h at $25 \pm 1^\circ\text{C}$. The concentrations used for EMS ranged from 0.1 to 0.4%, and from 0.01 to 0.04% for HZ. All solutions of the chemical mutagens were prepared in phosphate buffer of pH = 7. To facilitate uniform absorption, large quantities of solutions of mutagens, approximately three times the volume of seeds (Konzak *et al.*, 1965), were used. Following these treatments, the seeds were thoroughly washed in running

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tap water to reduce the residual mutagens sticking to the seed coat. The treated seeds were directly sown in the field along with untreated controls. Three replications of 100 seeds per treatment were sown in complete randomized block design (CRBD) to raise M_1 generation. The M_1 plants were harvested separately and the seeds sown the next season in plant progeny rows to raise M_2 generation. Distance between the seeds in a row and between rows was kept as 30 and 60 cm respectively. Chlorophyll mutants were scored in M_2 when seedlings were 8-20 days old and classified according to Gustafsson (1940). Frequency of chlorophyll mutants was calculated as percentage of M_2 segregating progenies as well as that of mutant seedlings in M_2 generation. Effectiveness and efficiency of different treatments of both mutagens were calculated by the method suggested by Konzak *et al.* (1965). Pollen fertility was estimated by staining the pollen grains with 1% acetocarmine solution. Pollen grains, which took the stain and had a regular shape, were considered fertile, and the shrunken, empty and unstained ones as sterile.

RESULTS AND DISCUSSION

In the present study, chlorophyll mutants were recorded in the field when seedlings were 8-20 days old. The spectrum of different chlorophyll mutants includes, *albina*, *xantha*, *chlorina* and *viriscent*. The seedlings, which initially appeared to be normal, began to manifest different types of chlorophyll deficiencies at the later stage of growth. All these chlorophyll deficient mutants were lethal except *viriscent*, which produced few seeds at maturity. A brief description of these chlorophyll mutants is given below:

Albina: Complete white leaves. A lethal mutant.

Xantha: Yellow to whitish yellow leaves and survived only upto 15-20 days.

Chlorina: Pale dull green-yellow to yellow-green and distinguished from *xantha* by the presence of green colour. Most of the seedlings showed mortality within 15 days.

Viriscent: Leaves with white or yellow patches. Plants were fertile and set seeds.

The *chlorina* mutants recorded the highest, followed by *albina*, *xantha* and *viriscent*. Occurrence of *chlorina* mutants in large number of crops have been attributed to different causes such as impaired chlorophyll biosynthesis, further degradation of chlorophyll and bleaching due to deficiency of carotenoids (Bevins *et al.*, 1992). Frequency of chlorophyll mutation was found to be concentration dependent and increased with increasing concentration of both mutagens under study (Table 1). The results are in concurrence with the findings of Khan *et al.* (1998) in mungbean. Chlorophyll mutations may result from alteration of the DNA in the chloroplasts and

EMS is known to react preferentially with guanine (Freese, 1963). EMS induced the highest frequency of chlorophyll mutations as compared to HZ (Table 1). This confirms that EMS is a more potent chemical mutagen in inducing chlorophyll mutants and supports the earlier report of Rao and Reddy (1985) in *Cajanus cajan* and Khan *et al.* (1998) in *Vigna radiata*.

Mutagenic effectiveness and efficiency

The mutagenic effectiveness as measured by the percentage of chlorophyll mutations divided by the unit dose of the mutagen was as high as 70.50 in EMS treatments, while the highest effectiveness of HZ was 600.00 (Table 2). HZ was found to be the most effective mutagen when compared to EMS. Both chemical mutagens were found to be more effective at low concentrations. Similar results were obtained by Prasad (1972), Waghmare and Mehra (2001) and Kharakwal (1998).

Mutagenic efficiency varied depending upon the criteria selected for estimation. In this study, the mutagenic efficiency, worked out on the basis of seedling injury (Mp/I), pollen sterility (Mp/S) and inhibition in seed germination (Mp/R), showed a decline with increasing concentration in all the treatments (Table 2). These results are in conformity to those reported earlier by Sharma (1977) and Khan and Siddiqui (1993). The greater efficiency at lower doses is because the biological damage generally increased with the enhancement in the dose at a higher rate than the mutations yielded in M_2 at the same dose (Konzak *et al.*, 1965). The mutagenic efficiency was more in EMS treated populations than those treated with HZ. The results indicate that the efficiency calculated on the basis of inhibition in seed germination was higher when compared to that based on seedling injury and pollen sterility. These results are in contrary to the earlier findings of Khan and Siddiqui (1992) in mungbean.

REFERENCES

- Ahmed John, S. 1999. Mutation frequency and chlorophyll mutations in parents and hybrid of cowpea following gamma irradiation. *Indian J. Genet.* 59(3): 357-361.
- Bevins, M., C.M. Yang and J. Markwell. 1992. Characterization of chlorophyll deficient mutant of sweet clover (*Melilotus alba*). *Pl. Physiol. and Biochem.* 30: 327-331.
- Das, P.K. and S. Kundagrami. 2000. Frequency and spectrum of chlorophyll mutations in grasspea induced by gamma rays. *Indian J. Genet.* 60(2): 239-241.
- Freese, E. 1963. Molecular mechanisms of mutations in molecular genetics. Pp. 207-269. Academic Press, New York.
- Gustafsson, A. 1940. The mutation system of chlorophyll apparatus. *Lunds Univ. Arsskr; M.F. Adv.*, 2Bd. 36 Nr. 11: 1-40.

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- Khan, S. and B.A. Siddiqui. 1992. Mutagenic effectiveness and efficiency of chemical mutagens in *Vigna radiata* (L.) Wilczek. Thai. J. Agric. Sci. 25: 291-297.
- Khan, S. and B.A. Siddiqui. 1993. Chlorophyll mutations in *Vigna radiata* (L.) Wilczek II. Mutagenic effectiveness and efficiency of chemical mutagens. Pak. J. Bot. 25(2): 161-166.
- Khan, S., M. Rehman, B.A. Siddiqui and S.A. Azad. 1998. Mutagen induced biological damage and chlorophyll mutations in *Vigna radiata* (L.) Wilczek. J. Ind. Bot. Soc. 77: 143-145.
- Kharakwal, M.C. 1998. Induced mutations in chickpea (*Cicer arietinum* L.).I. Comparative mutagenic efficiency and effectiveness of physical and chemical mutagens. Indian J. Genet. 58: 159-167.
- Konzak, C.F., R.A. Nilan, J. Wagner and R.J. Foster. 1965. Efficient chemical mutagenesis. Rad. Bot.(Suppl) 5: 49-70.
- Kumar, S. and D.K. Dubey. 1998. Mutagenic efficiency and effectiveness of separate and combined treatments with gamma rays, EMS and DES in Khesari (*Lathyrus sativus* L.). J. Ind. Bot. Soc. 77: 1-4.
- Prasad, M.V.R. 1972. A comparison of mutagenic effectiveness of gamma rays, EMS, NMU and NG. Indian J. Genet. 32: 360-367.
- Rao, D.M. and P.T. Reddy. 1985. Differential radio and chemosensitivity of pigeonpea (*Cajanus cajan* L.) varieties. Genet. Agr. 39: 153-161.
- Sharma, R.P. 1977. Induction of mutations for quantitative and qualitative characters in lentil (*Lens culinaris* L.). Ph.D. Thesis. IARI New Delhi.
- Singh, V.P., M. Singh and J.P. Pal. 1999. Mutagenic effects of gamma rays and EMS on frequency and spectrum of chlorophyll and macromutations in urdbean (*Vigna mungo* (L.) Hepper). Indian J. Genet. 59(2): 203-210.
- Waghmare, V.N. and R.B. Mehra. 2001. Induced chlorophyll mutations, mutagenic effectiveness and efficiency in *Lathyrus sativus* L. Indian J. Genet. 61(1): 53-56.

Table 1. Frequency and spectrum of chlorophyll mutants in M_1 generation of lentil var. Pant L-406.

<i>Treatment</i>	<i>No. of M_1 plant progenies</i>	<i>No. of plant progenies segregating in M_2</i>	<i>% Mutated plant progenies (Mp)</i>	<i>No. of M_1 plants</i>	<i>Chlorophyll mutant type</i>				<i>Total</i>	<i>Frequency (%)</i>
					<i>Albina</i>	<i>Xantha</i>	<i>Chlorina</i>	<i>Viriscent</i>		
Control	23	-	-	-	-	-	-	-		
0.1% EMS	26	11	42.30	85	5	4	4	2	15	5.26
0.2% EMS	24	14	58.33	270	6	8	5	1	20	7.40
0.3% EMS	27	17	62.96	315	7	6	9	2	24	7.62
0.4% EMS	25	16	64.00	338	7	6	11	3	27	7.98
0.01% HZ	25	09	36.00	294	6	3	2	1	12	4.08
0.02% HZ	27	14	51.85	327	4	7	5	2	18	5.50
0.03% HZ	28	16	57.14	338	5	5	8	2	20	5.92
0.04% HZ	26	16	61.54	354	7	6	9	2	24	-

EMS – Ethylmethane sulphonate, HZ – Hydrazine hydrate.

Table 2. Effectiveness and efficiency of EMS and HZ in M₂ generation of lentil var. Pant L-406.

Treatment	% Seedling injury (I)	% Pollen sterility (S)	% Inhibition in seed germination (R)	Mutagenic effectiveness (Mp/t.c)	Mutagenic efficiency		
					Mp/I	Mp/S	Mp/R
0.1% EMS	11.84	15.42	7.75	70.50	3.57	2.74	5.46
0.2% EMS	16.39	22.68	16.72	48.61	3.55	2.57	3.49
0.3% EMS	31.64	24.91	18.45	34.98	1.99	2.53	3.41
0.4% EMS	44.12	34.16	23.69	26.67	1.45	1.87	2.70
0.01% HZ	10.45	14.02	6.94	600.00	3.44	2.56	5.19
0.02% HZ	15.48	20.48	14.98	432.08	3.35	2.53	3.46
0.03% HZ	29.47	22.95	16.95	317.45	1.94	2.49	3.37
0.04% HZ	42.75	32.98	22.92	256.42	1.44	1.86	2.68